Original article

Dieckol attenuates the nociception and inflammatory responses in different nociceptive and inflammatory induced mice model

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ABSTRACT

Pain is the common indicator of inflammatory ailments and traumatic tissue injuries. The dieckol is an important therapeutic compound, which present in many seaweeds. The present research work was planned to assess the anti-inflammatory and anti-nociceptive actions of dieckol by using animal model. The anti-nociceptive action of dieckol was investigated by acetic acid triggered writhing, formalin provoked nociception, tail immersion test, hot-plate methods and the anti-inflammatory effects was explored by carrageenan triggered paw edema method. In the present investigation the administration of dieckol was remarkably suppressed and inhibited the acetic acid-provoked writhing, formalin-triggered nociception, tail immersion test, hot plate-provoked nociception in the experimental animals. The dieckol was significantly (p < 0.05) inhibited the carrageenan-triggered inflammation, leukocyte infiltration and diminished the formation of pro-inflammatory regulators in the experimental animals. Altogether, the dieckol was showed a potent anti-nociceptive and anti-inflammatory activity.

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1. Introduction

The sensation of pain is distinguished by the response to the inflammation which is induced by strong and deleterious stimuli and potential tissue damage. The pain is a primary response and indicator of tissue injuries (Bektas et al., 2015; IASP Taxonomy, 2017). There are so many types of pain were characterized and each was induced by the different kinds of stimuli and these pains were integrated with the serious physical discomfort which may directly affect the daily processes by unconstructively. To relive the pain there must be serious physiological efforts to be ensured such as, insufficient sleep during night, disintegration, nervousness and administration of immunosuppressive drugs (Helms and Barone 2008; Williams and Craig 2016). The pain is a primary

indication of inflammation and it is involving the sharing of various kinds of chemical arbitrators by molecular cascades which includes potassium ions and prostaglandins which could directly triggers the receptors of nociception (Wilson 2016; Gudes et al., 2015).

The inflammation is an immunological process and it is a response to the protective mechanisms of the body which is induced by the various kinds of obnoxious and harmful stimuli (Wang et al., 2014). This immunological process is a crucial mechanism and it involve in the various steps which includes extra vasations of plasma, migration of cellular mediators, vasodilation and the releasing of various mediators which is directly interlinked to the stimulation of pain. The inflammation is a common immunological process and known as the response to the protection against the damage of tissues and the invasion of the deleterious substances and microbes (Pomin, 2015). The process of inflammation was associated with the number of molecular level interactions (Pomin, 2015). The inflammation was playing a very imperative function in the pathological progress of several fatal ailments which includes rheumatoid arthritis, cardiovascular diseases and even the cancer (Kotas and Medzhitov, 2015).

The administration of non-steroidal anti-inflammatory drugs (NSAIDs) is a mostly utilized therapeutic method to the treat the pain. It gives a temporary relief from the pain and it often leads to several side effects and complications (Bruno et al., 2014). For

this reason, the urge to develop the novel herbal based anti-inflammatory and pain relieving herbal based drugs with the minimum or nil side effects and complications was received a significant attention among the researchers. The short time or long time administration of anti-inflammatory drugs can produce the serious immunological complications and side effects such as nausea, retention of urine and the reduction of the digestive system activity. Therefore, there is an urgent need to the development of novel pain killing drugs with improved efficiency and lesser of no side effects and complications (Krebs et al., 2016). Dieckol is a phlorotannin mainly presents in the marine alga *Ecklonia cava* that is largely exists in the Korean and Japanese seashores. Dieckol possessed the potent antioxidant (Li et al., 2009), anti-hepatic steatosis (Liu et al., 2019), anti-diabetic (Kang et al., 2013a), hepatoprotective (Kang et al., 2013b), and anti-ototoxic (Chang et al., 2016) activities. But still there are no any scientific literatures to claim the anti-nociceptive actions. The current exploration was planned to examine the anti-nociceptive and anti-inflammatory effects of dieckol by using several nociceptive and inflammation provoked animal model.

2. Materials and methods

2.1. Chemicals

All the chemicals and drugs which include dieckol, naloxone, diclofenac, glutamate, capsaicin, formalin, indomethacin and morphine was commercially purchased from the Sigma-Aldrich, United States of America.

2.2. Experimental animals

The male Swiss albino mice (20–30 g) was opted for the current exploration. All the experimental animals was maintained in clean plastic confines. The relative temperature was maintained between 22 and 25 °C with light and dark sequence of 12 h. The air moisture was maintained at the 55 to 60%. All the experimental animals were sustained for 14 days at laboratory situations in before the beginning of the investigations. After acclimatization the experimental animals were alienated into required groups for the experimentation.

2.3. Hot plate test

The hot plate induced anti-nociceptive action of the dieckol was scrutinized by method of Ankier, (1974). In before, the animals were used for the selection on a hot plate at 56 ± 07 °C. The animals that response at the time of latency time up to 16 s were selected for the experiment. After 24 h the male Swiss albino animal was supplemented the dieckol at of 5 mg/kg, 10 mg/kg and 20 mg/kg dosages. The morphine at the dose of 5 mg/kg was supplemented to the mice and served as a positive control. The reversal effects of naloxone were determined by administering the naloxone at 2 mg/kg with the dieckol at different doses (5 mg/kg to 20 mg/kg) and morphine (5 mg/kg). After the 60 min of the dieckol treatment and 30 min of the morphine treatment, the experimental mice was located on the surface of hot plate at the latency time. At the time, that the mice stayed on the hot plate without the flicking or licking of the hind limb/jumping was noted in seconds. The cut-off time for 30sec was set up to avert the damaging of tissue. The latency time of all the group of mice was measured at 30, 60, 90 and 120 min.

2.4. Acetic acid provoked writhing test

Acetic acid provoked writhing test were employed by the technique of Koster et al. (1959). The experimental mice was alienated into five groups 6 mice in each. The first group administered with normal water (control). The II, III and IV groups were supplemented with diverse concentration of dieckol at 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group V mice were administered with 10 mg/kg of diclofenac sodium. After the 60 min of the drug treatment all the experimental mice was supplemented with the 10 ml/kg of 0.6% acetic acid by intraperitoneally. After the five minutes of the acetic acid challenge, mice was were located in separate observation confines and writhing numbers in the abdominal region was measured for the each animal at the period of 10 min. The decreased number of the writhing in contrast to the control animals was considered as the proof of anti-nociception. The outcomes were depicted as the percentage of inhibition of writhing.

2.5. Formalin induced nociceptive test

The anti-nociceptive action of the dieckol by formalin test was employed by methods of Gomes et al. (2007). The experimental mice was alienated into five groups with 6 mice in each. The 10 ml/kg of normal water was supplemented to the first group of animals and assisted as control. The group II, III and IV was supplemented with the diverse concentration of dieckol 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group V mice was given with 10 mg/kg of morphine. After the 60 min of the drug treatment all the experimental animals were administered with the 20 μl of 1% formalin was given into the right side hind paw of the experimental animals by subcutaneously. The time in seconds which spending for the licking or biting of the formalin injected hind paw of animals was considered as the pointer of pain response. The response to the pain by the experimental animals were noted as two phases such as, first phase (5 min) and second phase (20 – 30 min) after the injection of formalin.

2.6. Tail immersion test

The tail immersion assay was executed by the technique of the Agrahari et al. (2010). The experimental animals were carefully controlled and the one third portion of the tail was immersed to the water bath at the temperature of 55 ± 3 °C. The mice which take out its tail within the 5 s were selected for the experiments. Every experimental animal was served with the control animal. The reaction time of the same group of animals were measured at the sequence of 30, 60, 90 and 120 min after the latency period of 60 min after the treatment of dieckol at the doses on 5 mg/kg, 10 mg/kg and 20 mg/kg. The control group given with 10 ml/kg of normal water and the positive control group received the 5 mg/kg of morphine. For the determination of reversal effects of naloxone, the 2 mg/kg of naloxone was served with the dieckol and morphine. During the experimentation, the cut off period for 10 s was noted for the avoidance of tail tissue damage.

2.7. Open field test

The open field assay were employed to determine the actions of dieckol on the natural loco motor activity of the experimental animals and this test was employed by the method prescribed by the De Mattos et al. (2007). The experimental mice was alienated into five groups and each group contains 6 animals. The first group was given with 10 ml/kg of normal water (control). The group II, III and IV was supplemented with the diverse concentration of dieckol 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group V mice

was given with the 10 mg/kg of morphine. After the treatment, the experimental animals were located at the center of the open field for 5 min gathering. During the session the square numbers walked by the all paws of the mice and immobility was noted.

2.8. Glutamate induced nociceptive test

The glutamate induced anti-nociceptive activity of dieckol was performed by technique of Beirith et al. (2002). The same experimental animals which is used in the acetic acid-provoked nociception assay was utilized this assay. The first group given with 10 ml/kg of normal water (control). The group II, III and IV was provided with diverse concentration of dieckol 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group V mice was given with the 10 mg/kg of diclofenac sodium. After the treatment with the respective drugs, the animals were injected with the 10 µl of glutamate at the right hind paw of the animals and then the animals was kept for the examination for 15 min. The total licking numbers showed by the animals was counted and noted.

2.9. Capsaicin induced nociceptive test

The effect of dieckol against the neuropathic nociception was determined by the technique of capsaicin-triggered nociceptive test described by the Luiz et al. (2007). The experimental mice was alienated into five groups with 6 mice in each. The 10 ml/kg of normal water was provided to the first group of animals and assisted as control. The group II, III and IV was provided with the diverse concentration of dieckol 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group V mice were given with 10 mg/kg of morphine. The treatment was done in 30 min prior to the nociception induction. After the supplementation, the left hind paw of the experimental animals was injected with the 1.6 µg/kg of capsaicin. After the induction the experimental animals were placed in the examination confines for 5 min and the total licking times of capsaicin administered paw was noted.

2.10. Carrageenan provoked paw edema test

The anti-inflammatory actions of dieckol was investigated by the carrageenan-provoked paw edema test which is elaborated by the Passos et al. (2007). The experimental animals was alienated into five groups with 6 mice in each. The 10 ml/kg of normal water were provided to the first group of animals and regarded as control. The group II, III and IV was supplemented with the diverse concentration of dieckol 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group V mice were given with 10 mg/kg of diclofenac sodium. The treatment was done in 60 min prior to the paw edema induction. After the supplementation, the right hind paw of the experimental mice was administered with the 50 ml of 1% carrageenan. After the injection the paw edema formation in mice were measured at 1 h interval for up to 4 h and the percentage of maximum possible effect (MPE) was measured.

2.10.1. Peritoneal cavity leukocyte infiltration test

After the induction of inflammation by injecting the carrageenan, the infiltration of leukocyte of peritoneal activity and the efficiency of dieckol against the carrageenan-triggered inflammation was determined by the technique of Vinegar et al. (1973). The experimental mice was alienated into five groups and each group contains 6 mice. The 10 ml/kg of normal water was supplemented to the first group of animals and regarded as control. The group II, III and IV was administered with the diverse concentration of dieckol 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group V mice was supplemented with the 10 mg/kg of morphine. The treatment was done in 30 min prior to the paw edema induc-

tion. After the administration, the right hind paw of the experimental animals was administered with the 500 µg of 1% carrageenan. After the 6 h of the experimentation, the mice were anesthetized with chloroform and sacrificed then the peritoneal cavity was collected and washed with the 2 ml of phosphate buffer which containing 1 mM EDTA to collect the leukocytes. The solution was then centrifuged at 3000 rpm for 50 min and the resulting suspension was subjected for the counting of total leukocytes and differential cells. The total leukocytes, mononuclear and polymorphonuclear cells was enumerated.

2.10.2. Analysis of pro-inflammatory cytokines level

The influence of dieckol on the pro-inflammatory mediators of the experimental animals were studied by the approach of Edwards et al. (1981). The mice was given to the mild chloroform anesthesia and then the back skin of the mice was shaved. Then the 5 ml of sterile distilled water was injected twice at the same spot by subcutaneously at the period of 3 days to develop a pouch. Then animals with the pouches were alienated into six groups and each group contains 6 mice. The 10 ml/g of 1% Tween 80 was administered to the first group and served as control. The second group received the 0.5 ml of carrageenan and the group III, IV and V were treated with the different concentration of dieckol 5 mg/kg, 10 mg/kg and 20 mg/kg respectively. The group VI mice were treated with the 10 mg/kg of Dexamethasone. After 1 h of experimentation, the animals were sacrificed, and then the pouch was cut to open and injected with the 2 ml of saline solution to the cavity and the same was sucked back to collect the cells. The collected suspension was centrifuged and resulting pellet was subjected to the examination of TNF-α, IL-6 and IL-1β.

2.11. Statistical analysis

The results were investigated by statistically by GraphPad Prism software (version 5.0) and the results were illustrated as mean ± SD. The one-way ANOVA successively the Dunnet's assay was employed to detect the significance between groups. The p value was regarded as $p < 0.05$ and $p < 0.01$.

3. Results

3.1. Anti-nociceptive activity of dieckol in hot plate induced nociception in mice

The observed results of hot plate provoked nociception method was clearly demonstrates that the dieckol was substantially elevated the latency time. The dieckol was showed an anti-nociceptive activity in dose dependent manner. The 5 mg/kg and 10 mg/kg of dieckol was displayed the remarkably increased inhibitory effects in 90 min and 120 min time. The 20 mg/kg of dieckol was showed a 20.36% of inhibitory effect in 30 min time whereas; it showed a maximum of 54.29% of nociception inhibitory effect in 120 min (Table 1). This effect was taken as maximum possible effect (% of MPE) which showed by the 20 mg/kg of dieckol. The administration of 5 mg/kg of morphine also displayed a substantial ($p < 0.01$) nociception inhibitory action (65.53%) in 120 min time. The administration of 2 mg/kg of naloxone along with the different concentration of dieckol was substantially ($p < 0.01$) reverted the nociceptive effects.

3.2. Anti-nociceptive activity of dieckol in tail immersion provoked nociception in mice

The results of tail immersion induced nociception method was exhibits that the dieckol was substantially ($p < 0.01$) augmented

Table 1
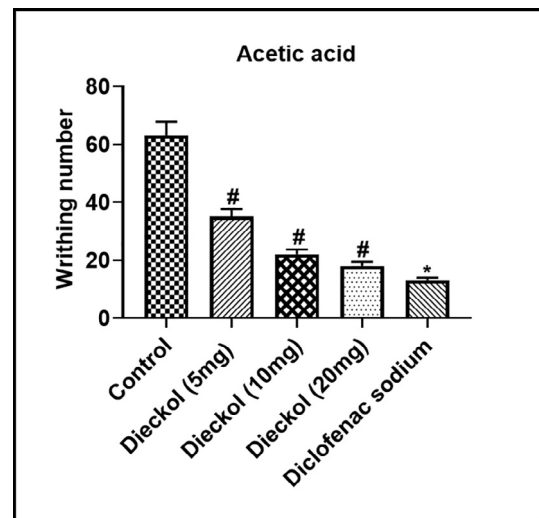Anti-nociceptive effect of dieckol in hot plate induced nociception mice model.

Treatment (mg/kg)	pre treatment	Response time(s)(%MPE)			
		30 min	60 min	90 min	120 min
Control	7.19 ± 0.10	7.26 ± 0.47	7.88 ± 0.37	7.97 ± 0.49	9.33 ± 0.53
Dieckol (5 mg)	7.82 ± 0.20	9.45 ± 0.26 (13.12)	11.70 ± 0.31 (21.86) [#]	11.97 ± 0.53 (33.81) [*]	12.27 ± 0.76 (36.43) [*]
Dieckol (10 mg)	7.33 ± 0.27	9.43 ± 0.48 (17.36)	11.69 ± 0.75 (29.14) [#]	12.44 ± 0.19 (33.21) [*]	12.76 ± 0.87 (42.37) [*]
Dieckol (20 mg)	7.89 ± 0.32	10.47 ± 0.19 (20.36)	12.78 ± 0.55 (42.47) [#]	13.74 ± 0.47 (51.76) [*]	14.77 ± 2.44 (54.29) [*]
Morphine (5 mg)	7.22 ± 0.74	12.75 ± 0.81 (39.17)	14.47 ± 0.29 (51.76) [#]	15.39 ± 0.37 (58.47) [*]	17.24 ± 1.47 (65.53)[*]
NLX (2 mg) + Control	7.57 ± 0.14	7.87 ± 0.63	8.24 ± 0.78	8.53 ± 0.76	8.87 ± 0.39
NLX (2 mg) + Dieckol (5 mg)	7.71 ± 0.17	8.38 ± 0.49 (9.14)	8.89 ± 0.83 (11.74) [#]	9.47 ± 0.76 (13.24) [*]	10.74 ± 0.82 (22.36) [*]
NLX (2 mg) + Dieckol (10 mg)	7.35 ± 0.47	7.94 ± 0.83 (10.27)	8.47 ± 0.37 (12.47) [#]	9.73 ± 0.69 (19.78) [*]	10.47 ± 0.48 (21.46) [*]
NLX (2 mg) + Dieckol (20 mg)	7.26 ± 0.47	7.76 ± 0.43 (11.33)	8.74 ± 0.75 (20.14) [#]	10.47 ± 0.47 (23.87) [*]	11.47 ± 0.27 (27.47) [*]
NLX (2 mg) + Morphine (5 mg)	7.57 ± 0.18	7.89 ± 0.59 (5.74)	9.57 ± 0.89 (13.57) [#]	10.47 ± 0.69 (20.69) [*]	13.47 ± 0.96 (33.47) [*]

Data were depicted as mean ± SD of 6 mice, [#], [*]illustrate statistical significance between control and treated groups at $p < 0.05$, $p < 0.01$, respectively using Dunnett's test.

the reaction time in the thermal stimuli. The dieckol was showed an anti-nociceptive activity against the tail immersion induced nociception. The 5 mg/kg and 10 mg/kg of dieckol was showed a significantly ($p < 0.01$) increased reaction time in the thermal pain stimuli in 90 min and 120 min time. The 20 mg/kg of dieckol was showed a 9.47% of inhibitory effect in 30 min time and it showed a maximum of 14.99% of tail immersion induced nociception inhibitory activity in 120 min time. This effect was taken as maximum possible effect (% of MPE) which showed by the 20 mg/kg of dieckol (table 2). The treatment with the 5 mg/kg of morphine showed a significant ($p < 0.01$) nociception inhibition activity (21.75%) in 120 min time. In this method also 2 mg/kg of naloxone administration along with the different concentration of dieckol was appreciably ($p < 0.01$) reverted the nociceptive effects.

3.3. Anti-nociceptive activity of dieckol in acetic acid induced nociception in mice

The results of acetic acid provoked nociception method was showed that the dieckol was remarkably ($p < 0.05$) diminished the acetic acid triggered nociception in the experimental mice. The administration of 5 mg/kg, 10 mg/kg and 20 mg/kg of dieckol was significantly ($p < 0.05$) diminished the writhing numbers in the experimental rats in contrast to the control (Fig. 1). The administration with the 20 mg/kg of dieckol was displayed a significantly suppressed the writhing numbers when compared to the 5 mg/kg and 10 mg/kg concentrations. The treatment with the diclofenac sodium was showed a substantially ($p < 0.01$) reduced writhing numbers when evaluated with control.

Fig. 1. Anti-nociceptive effect of Dieckol in the acetic acid induced nociception mice model Data were depicted as mean ± SD of 6 mice, [#], [*]illustrate statistical significance between control and treated groups at $p < 0.05$, $p < 0.01$, respectively using Dunnett's test.

3.4. Anti-nociceptive activity of dieckol in glutamate provoked nociception in mice

The results of glutamate provoked nociception method was showed that the dieckol was significantly ($p < 0.05$) diminished the glutamate triggered nociception in the experimental mice.

Table 2
Anti-nociceptive effect of dieckol in tail immersion induced nociception mice model.

Treatment (mg/kg)	pre treatment	Response time(s)(%MPE)			
		30 min	60 min	90 min	120 min
Control	6.19 ± 0.35	7.48 ± 0.14	7.69 ± 0.19	7.98 ± 0.26	6.69 ± 0.13
Dieckol (5 mg)	6.67 ± 0.29	8.47 ± 0.39 (4.96)	7.89 ± 0.45 (9.69)	7.93 ± 0.29 (9.24)	7.79 ± 0.82 (8.36)
Dieckol (10 mg)	6.14 ± 0.39	7.76 ± 0.74 (7.36)[#]	8.49 ± 0.36 (8.47)[*]	8.54 ± 0.17 (10.63) [*]	8.89 ± 0.82 (14.19)[*]
Dieckol (20 mg)	6.23 ± 0.49	6.38 ± 0.19 (9.47) [#]	8.29 ± 0.27 (11.96) [*]	9.39 ± 0.86 (13.12)[*]	9.96 ± 0.74 (14.99)[*]
Morphine (5 mg)	6.37 ± 0.19	8.58 ± 0.47 (11.74)[#]	8.89 ± 0.69 (17.39) [*]	9.76 ± 0.19 (19.36) [*]	9.87 ± 0.28 (21.75) [*]
NLX(2 mg) + Control	6.48 ± 0 0.19	6.68 ± 0.47	6.87 ± 0.74	7.58 ± 0.10	7.83 ± 0.87
NLX(2 mg) + Dieckol (5 mg)	6.69 ± 0.19	7.37 ± 0.47 (5.25) [#]	7.58 ± 0.39 (5.78) [*]	7.79 ± 0.19 (8.69) [*]	7.99 ± 0.48 (9.14) [*]
NLX(2 mg) + Dieckol (10 mg)	6.59 ± 0.14	9.28 ± 0.19 (5.67) [#]	9.78 ± 0.46 (6.26) [*]	9.93 ± 0.49 (7.96) [*]	9.49 ± 0.47 (8.96) [**]
NLX(2 mg) + Dieckol (20 mg)	6.92 ± 0.33	7.49 ± 0.86 (8.59) [#]	7.68 ± 0.37 (8.29) [*]	7.98 ± 0.59 (9.26) [*]	8.89 ± 0.36 (12.96) [*]
NLX(2 mg) + Morphine (5 mg)	6.47 ± 0.18	6.29 ± 0.27 (5.89) [#]	6.52 ± 0.58 (6.56) [*]	6.79 ± 0.69 (5.47) [*]	7.79 ± 0.41(11.96) [*]

Data were depicted as mean ± SD of 6 mice, [#], [*]illustrate statistical significance between control and treated groups at $p < 0.05$, $p < 0.01$, respectively using Dunnett's test.
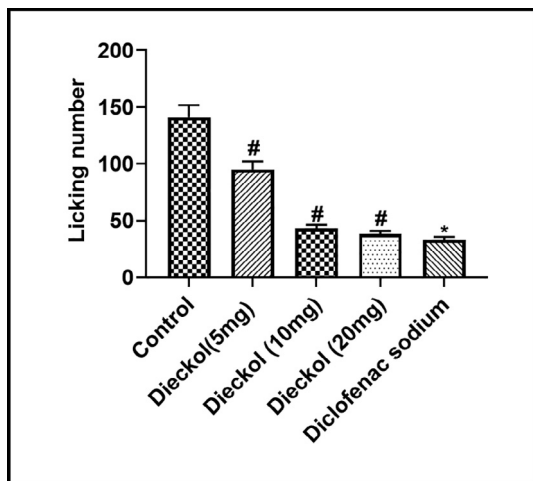
Fig. 2. **Anti-nociceptive effect of Dieckol in the glutamate induced nociception mice model** Data were depicted as mean ± SD of 6 mice, #, *illustrate statistical significance between control and treated groups at p < 0.05, p < 0.01, respectively using Dunnett's test.

The administration of 5 mg/kg, 10 mg/kg and 20 mg/kg of dieckol was significantly (p < 0.05) diminished the licking numbers when compared with control (Fig. 2). The treatment with the 20 mg/kg of dieckol was showed a significantly decreased number of lickings when compared to the 5 mg/kg and 10 mg/kg concentrations. The treatment with the diclofenac sodium was showed a significantly (p < 0.01) reduced number of lickings when compared with control.

3.5. Anti-nociceptive activity of dieckol in capsaicin induced nociception in mice

The dieckol was exhibited the significant (p < 0.05) anti-nociception action against the capsaicin provoked nociception in the experimental animals. The administration of 5 mg/kg, 10 mg/kg and 20 mg/kg of dieckol was appreciably (p < 0.05) suppressed the licking numbers when compared with control (Fig. 3).
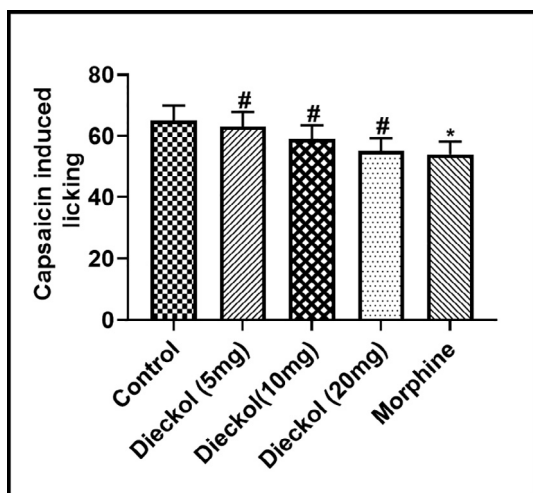
Fig. 3. **Anti-nociceptive effect of Dieckol in the capsaicin induced nociception mice model** Data were depicted as mean ± SD of 6 mice, #, *illustrate statistical significance between control and treated groups at p < 0.05, p < 0.01, respectively using Dunnett's test.

The treatment with the 20 mg/kg of dieckol was showed a significantly decreased number of lickings when compared to the 5 mg/kg and 10 mg/kg concentrations. The treatment with the diclofenac sodium was showed a substantially (p < 0.01) diminished the number of lickings when compared with control.

3.6. Anti-nociceptive activity of dieckol in formalin provoked nociception in mice

The results of formalin provoked nociception was showed that the dieckol was significantly (p < 0.05) diminished the glutamate provoked nociception in the experimental mice. The administration of 5 mg/kg, 10 mg/kg and 20 mg/kg of dieckol was remarkably (p < 0.05) suppressed the licking numbers when compared with control in both first phase and second phase (Fig. 4). The treatment with the 20 mg/kg of dieckol was showed a significantly decreased number of lickings when compared to the 5 mg/kg and 10 mg/kg concentrations in both phases. The reduction in licking is corresponding to the anti-nociceptive effects of dieckol. The supplementation of morphine was showed a remarkably (p < 0.01) diminished number of lickings when evaluated with control.

3.7. Anti-inflammatory activity of dieckol in carrageenan induced inflammation in mice

The results of anti-inflammatory action was exhibited that the dieckol was substantially diminished the paw volume which is increased by the carrageenan. The dieckol was showed an effective anti-inflammatory activity against the carrageenan triggered inflammation. The 5 mg/kg and 10 mg/kg of dieckol was showed a significantly (p < 0.01) reduced paw volume in 3rd hour and 4th hour time. The 20 mg/kg of dieckol was showed a 41.25% of inflammation inhibition in 1st hour and it showed a 42.96% of inflammation inhibition in 4th hour (Table 3). The treatment with the 10 mg/kg of indomethacin showed a substantial (p < 0.01) anti-inflammatory action (31.10%) in 4th hour.

3.8. Anti-inflammatory activity of dieckol against peritoneal leukocyte infiltration in carrageenan induced inflammation in mice

The results of leukocyte infiltration in carrageenan induced inflammation method was showed that the dieckol was significantly (p < 0.05) reduced the leukocyte, mononuclear and polynuclear infiltration which induced by the carrageenan in mice. The administration of 5 mg/kg, 10 mg/kg and 20 mg/kg of dieckol was remarkably (p < 0.05) suppressed the infiltration of leukocyte, mononuclear and polynuclear when compared with control (Fig. 5). The administration with the 20 mg/kg of dieckol was displayed the remarkably suppression in number of leukocyte, mononuclear and polynuclear infiltration when compared to the 5 mg/kg and 10 mg/kg concentrations of dieckol. The treatment with the diclofenac sodium was showed a significantly (p < 0.01) reduced number leukocyte, mononuclear and polynuclear infiltration when compared with control.

3.9. Anti-inflammatory activity of dieckol against pro-inflammatory cytokines in air pouch model in mice

The results of pro-inflammatory cytokines in air pouch mice model was showed that the dieckol was significantly (p < 0.05) diminished the pro-inflammatory regulators status which includes, TNF-α, IL-1β and IL-6 levels which increased by the carrageenan in mice. The supplementation of 5 mg/kg, 10 mg/kg and 20 mg/kg of dieckol was substantially (p < 0.05) diminished the pro-inflammatory regulators like TNF-α, IL-1β and IL-6 levels when compared to the control (Fig. 6). The administration of 20 mg/kg
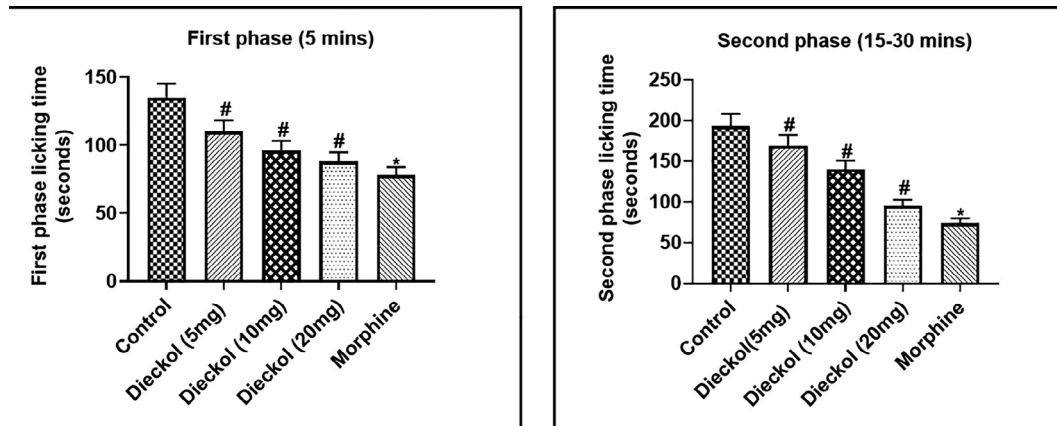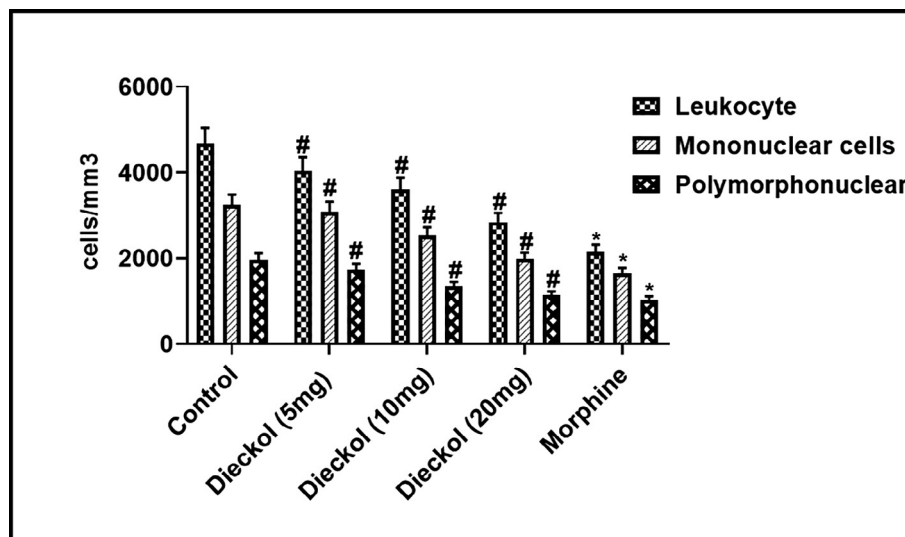
Fig. 4. Anti-nociceptive effect of Dieckol in biphasic formalin induced nociception mice model Data were depicted as mean ± SD of 6 mice, #, *illustrate statistical significance between control and treated groups at p < 0.05, p < 0.01, respectively using Dunnett's test.

Table 3
Anti-inflammatory effect of Dieckol in carrageenan induced inflammatory mice model.

Treatment (mg/kg)	Response time(s)(%MPE)				
	Basal	1st hour	2nd hour	3rd hour	4th hour
Control	52.89 ± 10.58	149.96 ± 9.25	136.96 ± 10.31	127.96 ± 10.01	101.67 ± 9.77
Dieckol (5 mg)	53.78 ± 11.73	88.744 ± 9.59 (41.14%)	86.69 ± 9.96 (40.15%)#	83.29 ± 9.47 (39.49%)*	75.34 ± 8.33 (29.26%)*
Dieckol (10 mg)	52.59 ± 4.28	84.74 ± 7.14 (39.45%)	79.47 ± 6.69 (36.28%) #	67.87 ± 5.63 (33.28%)*	64.87 ± 4.69 (31.23%)*
Dieckol (20 mg)	50.69 ± 6.75	86.36 ± 8.41 (41.25%)	72.876 ± 8.93 (42.33%)#	64.74 ± 6.12 (41.39%)*	61.39 ± 6.47 (42.96%)*
Indomethacin (10 mg)	54.83 ± 7.96	67.19 ± 7.86 (33.29%)	63.87 ± 6.84 (29.87%)#	69.86 ± 6.28 (32.56%) *	52.68 ± 6.87 (31.10%)*

Data were depicted as mean ± SD of 6 mice, #, *illustrate statistical significance between control and treated groups at p < 0.05, p < 0.01, respectively using Dunnett's test.

Fig. 5. Anti-inflammatory effect of Dieckol against peritoneal leukocyte infiltration in carageenan induced inflammatory mice model Data were depicted as mean ± SD of 6 mice, #, *illustrate statistical significance between control and treated groups at p < 0.05, p < 0.01, respectively using Dunnett's test.

of dieckol was showed a significantly reduced the pro-inflammatory mediators level like TNF-α, IL-1β and IL-6 levels when compared to the 5 mg/kg and 10 mg/kg concentrations of dieckol. The treatment with the diclofenac sodium was showed a significantly (p < 0.01) reduced the pro-inflammatory cytokines level like TNF-α, IL-1β and IL-6 status when compared with control.

3.10. Effects of dieckol on behaviour of mice in open field

The administration of dieckol does not show any disturbances on the locomotion of the experimental mice. There are very mild reduction in the locomotion of the mice was noted. The administration of 5 mg/kg, 10 mg/kg and 20 mg/kg of dieckol was significantly (p < 0.05) showed the very mild reduction in the walk-
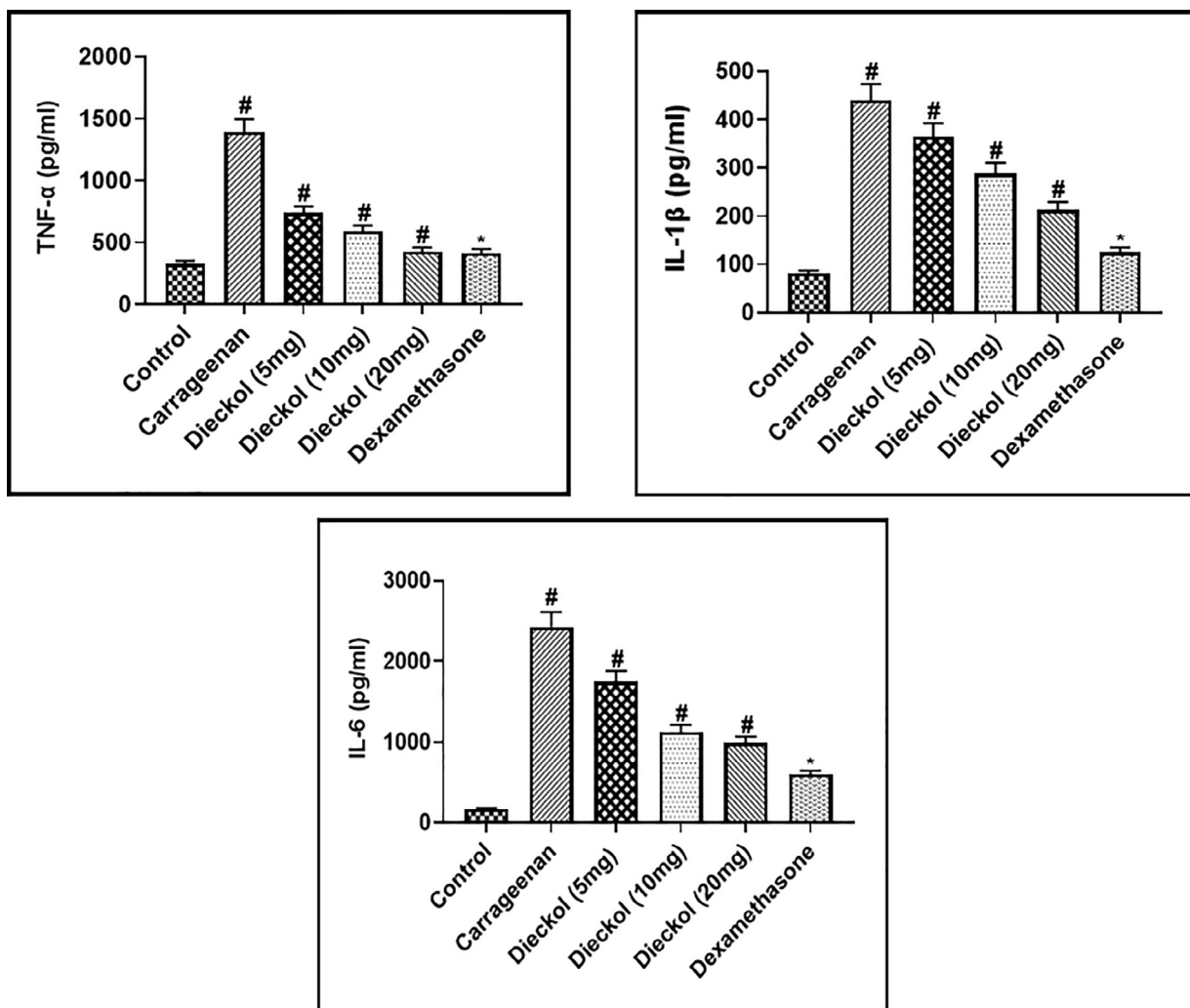
Fig. 6. Anti-inflammatory effect of Dieckol against pro-inflammatory cytokines in air pouch mice model Data were depicted as mean ± SD of 6 mice, #, *illustrate statistical significance between control and treated groups at $p < 0.05$, $p < 0.01$, respectively using Dunnett's test.

Fig. 7. Effect of Dieckol on behaviour of mice in open field Data were depicted as mean ± SD of 6 mice, #, *illustrate statistical significance between control and treated groups at $p < 0.05$, $p < 0.01$, respectively using Dunnett's test.
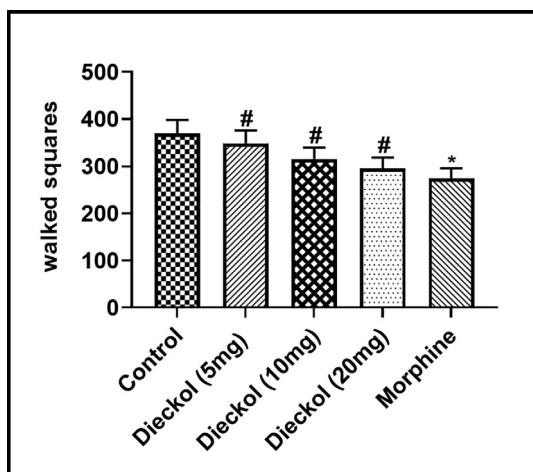
ing when compared with control (Fig. 7). The supllementation of 20 mg/kg of dieckol was displayed the very mild reduction walking of the mice when compared to the 5 mg/kg and 10 mg/kg concen-

trations. The treatment with the morphine was showed a significantly ($p < 0.01$) showed mild suppressed locomotion when compared with control.

4. Discussion

The current exploration was designed to examine the anti-nociceptive and anti-inflammatory actions of dieckol by using different nociception and inflammation induced animal model. The anti-nociceptive action of the dieckol was examined by using hot plate, tail immersion, acetic acid, glutamate, capsaicin and formalin provoked techniques. The anti-inflammatory activity of dieckol was determined by using carrageenan induced inflammation, peritoneal infiltration induced by carrageenan and estimating the pro-inflammatory regulators level in air pouch model. The nociception is characterized by an impulse reaction which is produced during the tissue damage and invading of external stimuli. The nociceptive experiment was employed to evaluate the remedial efficiency of drug and plant extracts to reduce the pain. These experiments can be executed by using mechanical, thermal or electrical stimuli (Le Bars et al., 2001). The acetic acid provoked abdominal writhing was one of the pain models which is widely executed to scrutinize the anti-nociceptive action of novel pain-relieving drugs and plant extracts (De Souza et al., 2009).

The acetic acid can stimulate the pain response by involving the intra-peritoneal release of various chemical mediators which includes histamine, acetyl choline-A, prostaglandins, neuromodulators and other neurotransmitters. These chemical mediators can increase the permeability of vascular and reduce the initiation of nociception (Gorzalczany et al., 2011; Pinheiro et al., 2012). In the current exploration the administration of dieckol was substantially (p < 0.01) diminished the writhing numbers in the abdomen of experimental mice when compared with control (Fig. 1). This finding was clearly exhibits that the dieckol has the anti-nociceptive activity. This effect of dieckol may be interlinked with the suppression of release of these inflammatory regulators to the cavity of peritoneal. This kind of anti-nociceptive action of the novel drugs may be due to the straight blocking of the pain mediating receptors which resulting in the anti-nociceptive effects (Loganayaki et al., 2012).

The formalin administration by subcutaneously to the mice can induce the biphasic (usually first phase and second phase) nociceptive response (Ramirez et al., 2010). The first phase of the nociception was directly corresponding to the neurogenic pain which is caused by the effects of formalin on the sensory fibers. The second phase of nociception also known as inflammatory phase was interlinked with the development of inflammatory reaction to release the nociceptive mediators which includes serotonin, bradykinin and prostaglandins (Reynoso et al., 2013). In the current exploration the administration of dieckol was substantially (p < 0.05) diminished the formalin provoked nociception in both first and second phases in contrast to the control in the experimental mice (Fig. 4). This result was demonstrates that the dieckol has the anti-nociceptive activity.

The tail immersion induced nociception model was one of the most used and very useful methods to define the anti-nociceptive activity of the novel drugs and plant extracts. The tail immersion induced pain model can be distinguishing the opiod like pain killers from the marginal pain killers (Le Bars et al., 2001). In the current exploration the treatment with the dieckol was remarkably (p < 0.01) augmented the latency time when compared with control. The dieckol administration was substantially (p < 0.01) increased the anti-nociceptive activity (Table 2). The effects of novel anti-nociceptive drugs or extracts can be determined by the method of open field test. This method was useful to study the any nonspecific disturbances of novel pain killing drugs on the locomotion of the experimental animals. In the current exploration, the supplementation of the dieckol was displayed the no any changes on the locomotion of the experimental animals or very mild changes was observed when compared with control (Fig. 7). This outcome was clearly showed that the dieckol has no any locomotor impairment of the animals and it showed a notable anti-nociceptive activity.

In the present research work the anti-inflammatory action of the dieckol was confirmed by using the technique of carrageenan provoked paw edema model in mice. The injection of carrageenan to the animals by subcutaneous method could enhance the development of pro-inflammatory mediators which can induce the signal of inflammation (Wang et al., 2014; Yi et al., 2010). The carrageenan administration into the hind paw of the experimental animals can promote the acute inflammation response by developing the paw edema. In the present research work the treatment with the dieckol was remarkably (p < 0.05) reduced and inhibited the carrageenan triggered inflammation in experimental animals. The mice which treated with the dieckol were showed the significantly reduced level of paw volume when compared to the carrageenan provoked mice. This outcome was clearly indicates that the dieckol has the effective anti-inflammatory activity.

The injection of carrageenan into the experimental animals can be resulting the generation of pro-inflammatory regulators which includes TNF-α, IL-1β and IL-6 that may promotes the deleterious inflammation. The TNF-α, IL-1β and IL-6 can be produced by the macrophages. The macrophages were work as key molecules which recruit the event of inflammation (Sergerie et al., 2007). The spinal glial cells have the ability to secrete the effective pro-inflammatory regulators like TNF-α, IL-1β and IL-6 and these cytokines could induce the receptors of pain (Schomberg and Olson 2012; Taves et al., 2013). In the current investigation the administration of dieckol was appreciably (p < 0.05) reduced the leukocyte, mononuclear and polynuclear infiltration which in induced by the carrageenan. The observed result of the present investigation was clearly indicates that the dieckol was exhibited a potent anti-nociceptive and anti-inflammatory action.

5. Conclusion

Based on the findings of the current research work, it was proved that the dieckol showed the potent anti-nociceptive and anti-inflammatory actions. The dieckol was effectively reduced the hot plate induced nociception, tail immersion induced nociception, glutamate, acetic acid and capsaicin induced nociception in mice. The dieckol also inhibited the carrageenan induced inflammation in experimental animals. Hence an overall outcome of the current investigation was clearly specifies that the dieckol has the potent anti-nociceptive and anti-inflammatory activity.

6. Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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